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(57) Abstract

The present invention provides modified methods and apparatus for the preparation of arrays of material wherein each array includes a preselected collection of polymers, small molecules or inorganic materials associated with a surface of a substrate. The methods of the invention provide for modifications to general apparatus, flow cell geometries and solutions used in array fabrication.

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ARRAY FABRICATION METHODS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of application Serial No. 09/019,881, filed February 6, 1998 and assigned to the assignee of the present invention.

BACKGROUND OF THE INVENTION

FIG. 1 illustrates a computerized system for forming and analyzing arrays of biological materials such as RNA or DNA. A computer 100 is used to design arrays of biological polymers such as RNA or DNA. The computer 100 may be, for example, an appropriately programmed Sun Workstation or personal computer or work station, such as an IBM PC equivalent, including appropriate memory and a CPU. The computer system 100 obtains inputs from a user regarding desired characteristics of a gene of interest, and other inputs regarding the desired features of the array. Optionally, the computer system may obtain information regarding a specific genetic sequence of interest from an external or internal database 102 such as GenBank. The output of the computer system 100 is a set of chip design computer files 104 in the form of, for example, a switch matrix, as described in PCT application WO 92/10092, and other associated computer files. PCT application WO 92/10092 is hereby incorporated by reference in its entirety for all purposes.

The chip design files are provided to a system 106 that designs the lithographic masks used in the fabrication of arrays of molecules such as DNA. The system or process 106 may include the hardware necessary to manufacture masks 110 and also the necessary computer hardware and software 108 necessary to lay the mask patterns out on the mask in an efficient manner. As with the other features in FIG. 1, such equipment may or may not be located at the same physical site, but is

shown together for ease of illustration in FIG. 1. The system 106 generates masks 110 such as chrome-on-glass masks for use in the fabrication of polymer arrays.

The masks 110, as well as selected information relating to the design of the chips from system 100, are used in a synthesis system 112. Synthesis system 112 includes the necessary hardware and software used to fabricate arrays of polymers on a substrate or chip 114. For example, synthesizer 112 includes a light source 116 and a chemical flow cell 118 on which the substrate or chip 114 is placed. Mask 110 is placed between the light source and the substrate/chip, and the two are translated relative to each other at appropriate times for deprotection of selected regions of the chip. Selected chemical reagents are directed through flow cell 118 for coupling to deprotected regions, as well as for washing and other operations. All operations are preferably directed by an appropriately programmed digital computer 119, which may or may not be the same computer as the computer(s) used in mask design and mask making.

The substrates fabricated by synthesis system 112 are optionally diced into smaller chips and exposed to marked receptors. The receptors may or may not be complementary to one or more of the molecules on the substrate. The receptors are marked with a label such as a fluorescein label (indicated by an asterisk in FIG. 1) and placed in scanning system 120. Scanning system 120 again operates under the direction of an appropriately programmed digital computer 122, which also may or may not be the same computer as the computers used in synthesis, mask making, and mask design. The scanner 120 includes a detection device 124 such as a confocal microscope or CCD (charge-coupled device) that is used to detect the location where labeled receptor (*) has bound to the substrate. The output of scanner 120 is an image file(s) 124 indicating, in the case of fluorescein labeled receptor, the fluorescence intensity (photon counts or other related measurements, such as voltage) as a function of position on the substrate. Since higher photon counts will be observed where the labeled receptor has bound more strongly to the array of polymers, and since the monomer sequence of the polymers on the substrate is known as a function of position, it becomes possible to determine the sequence(s) of polymer(s) on the

substrate that are complementary to the receptor.

The image file 124 is provided as input to an analysis system 126. Again, the analysis system may be any one of a wide variety of computer system(s), but in a preferred embodiment the analysis system is based on a Sun Workstation or equivalent. Using information regarding the molecular sequences obtained from the chip design files and the image files, the analysis system performs one or more of a variety of tasks. In one embodiment the analysis system compares the patterns of fluorescence generated by a receptor of interest to patterns that would be expected from a "wild" type receptor, providing appropriate output 128. If the pattern of fluorescence matches (within limits) that of the wild type receptor, it is assumed that the receptor of interest is the same as that of the wild type receptor. If the pattern of fluorescence is significantly different than that of the wild type receptor, it is assumed that the receptor is not wild type receptor. The system may further be used to identify specific mutations in a receptor such as DNA or RNA, and may in some embodiments sequence all or part of a particular receptor de novo.

FIG. 2A provides a simplified illustration of the software system used in conjunction with the system for forming and analyzing arrays shown in Figure 1. As shown in FIG. 2A, the system first identifies the genetic sequencer(s) that would be of interest in a particular analysis at step 202. The sequences of interest may, for example, be normal or mutant portions of a gene, genes that identify heredity, provide forensic information, or the like. Sequence selection may be provided via manual input of text files or may be from external sources such as GenBank. At step 204 the system evaluates the gene to determine or assist the user in determining which probes would be desirable on the chip, and provides an appropriate "layout" on the chip for the probes. The layout will implement desired characteristics such as minimization of edge effects, ease of synthesis, and/or arrangement on the chip that permits "reading" of genetic sequence.

At step 206 the masks for the synthesis are designed. Again, the masks will be designed to implement one or more desired attributes. For example, the masks may be designed to reduce the number of masks that will be needed, reduce the

number of pixels that must be "opened" on the mask, and/or reduce the number of exposures required in synthesis of the mask, thereby reducing cost substantially.

At step 208 the software utilizes the mask design and layout information to make the DNA or other polymer chips. This software 208 will control, among other things, relative translation of a substrate and the mask, the flow of desired reagents through a flow cell, the synthesis temperature of the flow cell, and other parameters. At step 210, another piece of software is used in scanning a chip thus synthesized and exposed to a labeled receptor.

The software controls the scanning of the chip, and stores the data thus obtained in a file that may later be utilized to extract sequence information.

At step 212 the software system utilizes the layout information and the fluorescence information to evaluate the chip. Among the important pieces of information obtained from DNA chips are the identification of mutant receptors, and determination of genetic sequence of a particular receptor.

FIG. 2B illustrates the binding of a particular target DNA to an array of DNA probes 114. As shown in this simple example, the following probes are formed in the array:

3'-AGAACGT
AGAACGA
AGAACGG
AGAACGC
.
.
.

When a fluorescein-labeled (or other marked) target with the sequence 5'-TCTTGCA is exposed to the array, it is complementary only to the probe 3'-AGAACGT, and fluorescein will be found on the surface of the substrate where 3'-AGAACGT is located. By contrast, if 5'-TCTTGCT is exposed to the array, it will bind only (or most strongly) to 3'-AGAACGA. By identifying the location where a target hybridizes to the array of probes most strongly, it becomes possible to extract sequence information from such arrays using the invention herein.

New technology, called VLSIPS , has enabled the production of chips smaller than a thumbnail that contain hundreds of thousands or more of different molecular probes. These techniques are described in U.S. Pat. No. 5,143,854, PCT WO 92/10092, and PCT WO 90/15070, which are herein incorporated by reference in their entirety for all purposes. In practice, biological chips have probes arranged in arrays, each probe ensemble assigned a specific location. Biological chips have been produced in which each location has a scale of, for example, ten microns. As noted above, the chips can be used to determine whether target molecules interact with any of the probes on the chip. After exposing the array to target molecules under selected test conditions, scanning devices can examine each location in the array and determine whether a target molecule has interacted with the probe at that location.

Biological chips are useful in a variety of screening techniques for obtaining information about either the probes or the target molecules. For example, a library of peptides can be used as probes to screen for drugs. The peptides can be exposed to a receptor, and those probes that bind to the receptor can be identified.

For example, biological chips wherein the probes are oligonucleotides ("oligonucleotide arrays") show promise. Arrays of nucleic acid probes can be used to extract sequence information from nucleic acid samples. The samples are exposed to the probes under conditions that allow hybridization. The arrays are then scanned to determine to which probes the sample molecules have hybridized. One can obtain sequence information by selective tiling of the probes with particular sequences on the arrays, and using algorithms to compare patterns of hybridization and non-hybridization. This method is useful for sequencing nucleic acids. It is also useful in diagnostic screening for genetic diseases or for the presence of a particular pathogen or a strain of pathogen.

Methods and apparatus for synthesizing a variety of different types of polymers are well known in the art. For example, the "Merrifield" method,

described in Atherton et al., "Solid Phase Peptide Synthesis," IRL Press, 1989, which is incorporated herein by reference for all purposes, has been used to synthesize peptides on a solid support. In the Merrifield method, an amino acid is covalently bonded to a support made of an insoluble polymer. Another amino acid with an alpha protected group is reacted with the covalently bonded amino acid to form a dipeptide. After washing, the protective group is removed and a third amino acid with an alpha protective group is added to the dipeptide. This process is continued until a peptide of a desired length and sequence is obtained.

It has also been proposed to use a series of reaction vessels for polymer synthesis. For example, a tubular reactor system may be used to synthesize a linear polymer on a solid phase support by automated sequential addition of reagents.

Methods of preparing a plurality of polymer sequences are also known in which a porous container encloses a known quantity of reactive particles, the particles being larger in size than pores of the container. The containers may be selectively reacted with desired materials to synthesize desired sequences of product molecules.

Other techniques have also been described. These methods include the synthesis of peptides on 96 plastic pins which fit the format of standard microtiter plates.

Methods have also been developed for producing large arrays of polymer sequences on solid substrates. These large "arrays" of polymer sequences have wide ranging applications and are of substantial importance to the pharmaceutical, biotechnology and medical industries. For example, the arrays may be used in screening large numbers of molecules for biological activity, e.g., receptor binding capability. Alternatively, arrays of nucleic acid probes can be used to identify mutations in known sequences. Of particular note, is the pioneering work described in U.S. Patent No. 5,445,934 (Fodor et al.) and U.S. Patent No. 5,510,270 (Fodor et al.) which disclose improved methods of molecular synthesis using light directed techniques and are hereby incorporated by reference in their

entirety for all purposes.

The present invention is directed to modified techniques and methods for use in the process of array fabrication as described above.

SUMMARY OF THE INVENTION

The present invention provides modified methods and apparatus for the preparation of arrays of material wherein each array includes a preselected collection of polymers, small molecules or inorganic materials associated with a surface of a substrate. In one embodiment of the invention, a method of removing static charge during the fabrication of an array is described. The method of static charge removal may include, but is not limited to, the use of an ionizing fan or ion bars. In particular, equipment for removing static charges is placed at each entry and exit point in the flow cell. In another embodiment of the invention, optimized flow cell geometries are provided. In another embodiment of the invention, the introduction of phosphoramidite during array fabrication is provided. In another embodiment of the invention, a novel deprotection solution is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the overall system and method of operation for array fabrication.

Figure 2A is an illustration of the overall operation of the software involved in the system of Figure 1.

Figure 2B illustrates conceptually the binding of probes on chips.

Figure 3 schematically illustrates a reactor system for carrying out the combined photolysis / chemistry steps of the present invention;

Figure 4 schematically illustrates a reactor system in which static removal devices are placed at entry and exit points on the reactor;

Figure 5 illustrates the mechanism whereby a substrate is moved through a flow cell;

Figure 6 depicts a cross-section of a flow cell design wherein shims are machined into the surface of the flow cell; and

Figure 7 schematically illustrates the locations of the electrostatic field measurements.

DEFINITIONS

Array: An array is a preselected collection of different polymer sequences, small molecules or inorganic materials which are associated with a surface of a substrate. An array may include polymers of a given length having all possible monomer sequences made up of a specific basis set of monomers, or a specific subset of such an array. In other cases an array may be formed from inorganic materials (See Schultz et al PCT application WO 96/11878, which is hereby incorporated by reference in its entirety for all purposes.)

Monomer: A member of the set of small molecules which can be joined together to form a polymer. The set of monomers includes but is not restricted to, for example, the set of common L-amino acids, the set of D-amino acids, the set of natural or synthetic amino acids, the set of nucleotides and the set of pentoses and hexoses. As used herein, monomer refers to any number of a basis set for synthesis of a polymer. For example, dimers of the 20 naturally occurring L-amino acids form a basis set of 400 monomers for synthesis of polypeptides. Different basis sets of monomers may be used in any of the successive steps in the synthesis of a polymer. Furthermore, each of the sets may include protected members which are modified after synthesis.

Substrate: A material having a rigid or semi-rigid surface. In many embodiments, at least one surface of the substrate will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different polymers with, for example, wells, raised regions, etched trenches, or the like. According to other embodiments, small beads may be provided on the surface which may be released upon completion

of the synthesis.

Protective Group: A material which is bound to a monomer unit and which may be selectively removed therefrom for exposure of a reactive group. A protective group generally prevents undesired reactions from taking place (such as coupling) until such time as the protective group is removed.

Reactive Group: Refers to a portion of a molecule which, under selected circumstances performs a desired coupling or cleavage reaction with another moiety. Such coupling may be via covalent or other types of bonds.

DESCRIPTION OF THE PRESENT INVENTION

Methods for synthesis of arrays of material have been previously described. For example, methods of synthesizing arrays of large numbers of polymer sequences, including oligonucleotide and peptides, on a single substrate have been described. See U.S. patent Nos. 5,143,854 and 5,384,261 and published PCT Application No. WO 92/10092, each of which is incorporated herein by reference in its entirety for all purposes. Methods for synthesizing arrays of inorganic materials have also been described. See published PCT Application No. WO 96/11878.

As described previously, the synthesis of materials on the surface of a substrate may be carried out using light directed methods as described in., e.g. U.S. Patent Nos. 5,143,854 and 5,384,261 and Published PCT Application No. WO 92/10092, or mechanical synthesis methods as described in 5,384,261 and Published PCT Application No. WO 93/09668, each of which is incorporated herein by reference. In one embodiment, synthesis is carried out using light-directed synthesis methods. In particular, these light-directed or photolithographic synthesis methods involve a photolysis step and a chemistry step. Briefly, the substrate surface comprises functional groups on its surface. These functional groups are protected by photo labile protecting groups ("photoprotected"). During the photolysis step, portions of the surface of the

substrate are exposed to light or other activators to activate the functional groups within those portions, i.e., to remove photoprotecting groups. The substrate is then subjected to a chemistry step in which chemical monomers that are photoprotected at least one functional group are then contacted with the surface of the substrate. These monomers bind to the activated portion of the substrate through an unprotected functional group.

Subsequent activation and coupling steps couple monomers to the other preselected regions, which may overlap with all or part of the first region. The activation and coupling sequence at each region on the substrate determines the sequence of the polymer synthesized thereon.

While light directed techniques are described herein by way of example, the inventions herein will have application to other technologies such as ink jet or flow cell synthesis methods see Winkler et al, U.S. Pat. No. 5,384,261, and through the use of applied electrical fields see Fodor et al, U.S. Pat. No. 5,143,854, or even placement of presynthesized materials on a support by the above or other methods. See also co-pending U.S. Serial No.08/634,053, each of which is incorporated herein by reference in its entirety for all purposes.

The equipment involved in array fabrication can, particularly at certain times of the year, have a tendency to build up an inconsistent electrostatic charge, which can be either positive or negative. This electrostatic charge can, in some cases impact the consistency and quality of fabricated arrays. It is believed that this charge may be caused primarily by weather fluctuations, however, regardless of the cause, the removal or variation of the electrostatic charge can be beneficial to the fabrication process. Accordingly, the present invention provides, in one embodiment, for the removal of electrostatic charge from the fabrication process. This removal of electrostatic charge may be accomplished, for example, by the addition of ionizing fans, either large fans embedded in the ceiling above the equipment or small fans placed in areas which are recorded to have levels of electrostatic charge. In some particular cases, this

electrostatic charge may be manipulated to enhance the performance of array fabrication, either in terms of a reduced length of time required for fabrication, or in an increased yield.

In one particular example of fabrication of arrayed polymers through light-directed synthesis, the substrate preparation process combines the photolysis and chemistry steps in a single unit operation. The substrate wafer is mounted in a flow cell during both the photolysis and chemistry or monomer addition steps. In particular, the substrate is mounted in a reactor system that allows for the photolytic exposure of the synthesis surface of the substrate to activate the functional groups thereon. Solutions containing chemical monomers are then introduced into the reactor system and contacted with the synthesis surface, where the monomers can bind with the active functional groups on the substrate surface. The monomer containing solution is then removed from the reactor system, and another photolysis step is performed, exposing and activating different selected regions of the substrate surface. This process is repeated until the desired polymer arrays are created.

A schematic illustration of a device for carrying out the combined photolysis/chemistry step of the individual process is shown at Figure 3. The device of Figure 3 corresponds to chemical flow cell 118 in Figure 1. The figure shows a cross-sectional view of the reactor system 300. The device includes a flow cell which is made up of a body 302 having a cavity 304 disposed in one surface. The cavity generally includes fluid inlets 308 and outlets 310 for flowing fluid into and through the cavity. The cavity may optionally include ridges 306 on the back surface of the cavity to aid in mixing the fluids as they are pumped into and through the cavity. The substrate 312 is mounted over the cavity whereby the front surface of the substrate wafer 314 (the surface upon which the arrays are to be synthesized) is in fluid communication with the cavity. The device also includes a fluid delivery system in fluid connection with the fluid inlet 308 for delivering selected fluids into the cavity to contact the first

surface of the substrate. The fluid delivery system typically delivers selected fluids, e.g. monomer containing solutions, index matching fluids, wash solutions, etc., from one or more reagent reservoirs 318, into the cavity via the fluid inlet 308. The delivery system typically includes a pump 316 and one or more valves 317 to select from the various reagent reservoirs. Aspects of this invention are described in further detail in co-pending application Serial No. 08/634,053 which is incorporated herein for all purposes.

According to one preferred embodiment, an electrostatic charge removal device 322 is placed in contact or in proximity with the reactor system 300 for active removal of the charge. In one embodiment, this electrostatic removal device is a small portable electrostatic fan, in another embodiment it is a large fan embedded in the ceiling above the reactor system. In yet another embodiment, a charged static bar may be incorporated into a system wherein the ions from the bar are blown across the fabrication system by a nonelectrostatic fan. Other means of removing the electrostatic charge from the fabrication device will be apparent to one skilled in the art and this disclosure is not intended to be limited to the above named methods.

According to the present invention, as shown in Figure 4, electrostatic charge removal devices 322 are preferrably placed at the entry and exit points of the flow cell 302. Figure 4 shows entry point 400 and exit point 401 on flow cell 302. Such exit and entry points 400, 401 may be in the form of doors, for example. However, a variety of entry and exit points may be employed in a given flow cell and may be physically constructed in a variety of ways. According to this embodiment of the present invention, any entry and exit point on the flow cell is provided with an electrostatic charge removal device 322 at its proximity. Such devices 322 can be electrostatic dissipative fans, charged static bars, or a combination of both. By placing electrostatic charge removal devices 322 at the entry and exit points of the flow cell, undesired static is eliminated and any possible effect on the chemistry that occurs in the flow cell

where the arrays are built can be avoided. In addition to affecting the chemistry occurring in the flow cell, static electricity may cause the substrates to stick to the flow cell and thus causes problems in moving the substrates through the process. Placing the electrostatic charge removal devices 322 at the entry and exit points of the flow cell thus shower the facility to provide a focussed dissipative effect. This ensures that the substrate is charge neutral before entering the flow cell and before beginning the chemistry within the flow cell. In addition, the substrate is charge neutral so that the substrate does not physically stick to the door or any other portion of the flow cell when moved.

Figure 5 shows a cross-sectional view of the deice for moving the substrate through the flow cell. Typically, the flow cell includes metal pins 520 that push the substrate 514 so that a robotic handler can pick up the substrate after processing within the flow cell. If static were allowed to collect such that the substrate was physically stuck to, for example, the door or other portion of the flow cell, the pins used to push the substrate from the flow cell may cause the substrate to break the substrate.

In another aspect of the present invention, the metal pins 520 used to push the substrate 514 up and off of the door, as described above, are preferably covered with plastic or Teflon tips. It was found that when uncovered metal pins were used to extricate the substrate, a capacitive effect was occurring which caused a visible undesired effect on the wafer after synthesis. Covering the metal pins with plastic, Teflon or some other non-conducting material avoids any possibility of charge passing from the pin to the back of the wafer when the pins are pushed up to move the wafer or during synthesis (i.e., when the door of the flow cell is closed).

The present invention also provides design modifications to the flow cell. In one embodiment of the invention the flow cell body is designed in such as way as to form a contiguous body on which the substrate may fit while still allowing for the formation of a tight seal between the substrate and the flow cell

body. It is believed that this design will prevent cracking of the substrate during the fabrication process. In one embodiment of the invention a shim often enclosing an o-ring is machined into the surface of the flow cell to form one contiguous surface. Figure 6 depicts a cross-section of one embodiment of the flow cell design. A groove 601 is cut into the flow cell body 302 having a cavity 304. The shim 602, containing an o-ring 604, forms a tight seal between the body and the substrate 312 thereby creating a flat, contiguous surface where the substrate contacts the flow cell body.

It is often desirable to maintain the volume of the flow cell cavity as small as possible so as to more accurately control reaction parameters such as temperature or concentration of chemicals. However, flow cell cavities which are too small, as in a flow cell with a working depth of 0.010" or less for a flow cell measuring 5" in length and 5" in width, may lead to reduced yield by trapping bubbles from the reaction fluids in the cavity resulting in incomplete exposure of the substrate surface to the reaction fluid. It is important that the reagents in the flow cell cavity be allowed to mix completely. Flow cell cavities with an insufficient depth to length/width ratio may also interfere with complete mixing due to surface tension created by inadequate reaction volume size. Due to this factor, appropriate reaction cavity depth will vary with the width and length of the flow cell cavity. For a flow cell measuring 5" x 5", preferred reaction cavity depths are 0.100" working depth to 0.005" working depth and more preferably 0.050" to 0.005", more preferably 0.032" to 0.010" with an 0.020" working depth being the most preferred.

In another aspect of the present invention, the material construction of the flow cell is discussed. Typically, within the chamber of the flow cell, one surface is glass which is formed by the wafer itself. The other surface of the flow cell may be one of several materials, including stainless steel or glass.

In an embodiment employing stainless steel, the stainless steel material is preferably passited and electropolished for smoothness which enhances the

mixing characteristics within the flow cell. Stainless steel is cost effective and easy to machine. However, because stainless steel is not perfectly chemically inert, glass is preferably employed as the second surface of the flow cell. In particular, borosilicate glass is useful as it is chemically inert. However, any chemically inert glass may be used as long as it does not easily contaminate, corrode, or degrade the substrate. Borosilicate glass also provides an advantage over stainless steel in that it provides the smoothest possible surface, again which provides for enhanced mixing characteristics for chemicals.

With the borosilicate glass as the second surface of the flow cell, everything in the chamber is glass, except for the O-ring seals that seal the perimeter. As a result, there is no material inside the flow chamber that might corrode, oxidize or otherwise degrade the chemical reactions going on inside.

In light directed synthesis, a monomer building block is introduced or contacted with the synthesis surface of the substrate following each photolysis step. The added monomer often includes a single active functional group, for example, in the case of oligonucleotide synthesis, a 3'-hydroxyl group. The remaining functional group that is involved in linking the monomer within the polymer sequence, e.g., the 5'-hydroxyl group of a nucleotide, is generally photoprotected. The monomers then bind to the reactive moieties on the surface of the substrate, activated during the preceding photolysis step, or at the termini of linker molecules of polymers being synthesized on the substrate.

The chemistry step often involves solid phase polymer synthesis methods that are well known in the art. For example, detailed descriptions of the procedures for solid phase synthesis of oligonucleotide by phosphoramidite, phosphite-triester, phosphotriester, and H-phosphonate chemistries are widely available. *See* Gait, ed. *Oligonucleotide Synthesis: A Practical Approach,* IRL Press, Washington D.C. (1984) which is incorporated herein by reference for all purposes.

In one embodiment of the invention, a solution containing a 3'-0-

activated phosphoramidite nucleoside, photoprotected at the 5' hydroxyl is introduced into the flow cell for coupling to the photo activated regions of the substrate. Typically the phosphoramidite nucleoside is present in the monomer solution at a concentration of from mm to about 100mM, more preferably at 10mM to about 50mM, more preferably at 15mM to about 30mM and most preferably at a concentration of about 20mM.

Following overall synthesis of the desired polymers on the substrate wafers, permanent protecting groups, e.g., those which were not removed during each synthesis step, typically remain on nucleobases and the phosphate backbone of synthetic oligonucleotide. Removal of these protecting groups is usually accomplished with a concentrated solution of aqueous ammonium hydroxide. While this method is effective for the removal of the protecting groups, these conditions can also result in some amount of cleavage of the synthetic oligomers from the support (usually porous silica particles) by hydrolyzing an ester linkage between the oligo and a functionalized silane derivative that is bonded to the support. In arrays, it is desirable to preserve the linkage connecting the oligonucleotide to the substrate after the final deprotection step. For this reason, synthesis is carried out directly on the substrate which is derivatized with a hydroxylalkyl-trialkoxysilane (e.g., bis(hydroxyethyl)aminopropylsilane). However, these supports are not completely stable to the alkaline hydrolysis conditions used for deprotection. Depending upon the duration, substrates left in aqueous ammonia for protracted periods can suffer a loss of probes due to hydroxide ion attack on the silane bonded phase.

Co-pending application No. 08/634,053, which is incorporated herein for all purposes, describes final deprotection of the polymer sequence using anhydrous organic amines. In particular, primary and secondary alkylamines are used to effect final deprotection. The alkylamines may be used undiluted or in a solution of an organic solvent, e.g. ethanol, acetonitrile, or the like.

One embodiment of the present invention provides that the active ingredient in the solution be diluted in water. Preferred embodiments include, but are not limited to, Methylamine[MET]/H_2O, Ethylenediamine[EDA]/H_2O, Ethanolamine[ETA]/H_2O, and Ammonia Hydroxide/H_20. Typically the solution of alkylamine will be at least about 40% alkylamine (v/v). Depending upon the protecting groups to be removed, the time required for complete deprotection in these solutions ranges from several minutes for "fast" base-protecting groups, e.g. PAC or DMF-protected A, C or G and Ibu-protected C, to from, for example, 4 to 20 hours for the standard protecting groups, e.g. benzoyl-protected A, C, or G and Ibu-protected G

EXAMPLES

Temporary ionizing fans were first installed on or nearby areas where highly variable electrostatic charges were detected on the array fabrication equipment. Table 1 shows electrostatic field intensity measurements taken before ionization emitter fans were installed in the clean room pods. All measurements were taken with a Model 775PVS fieldmeter from Ion Systems, Inc.

Table 1
Measurement of static charge on apparatus (kV/inch)

Location	Apparatus # A	B	C		Time of Measurement
Substrate wafer on load paddle hours before process (position 1)	+6.00		-4.00		Time 1
	+8.00		-0.05	-4.00	Time 1+ 5
Substrate wafer on unload paddle hours after process (position 2)	+7.00		-4.00		Time 1
	+10.0		-0.40	-3.00	Time 1 + 5
Flowcell door glass without hours substrate wafer before process (position 3)	-0.30	+0.10			Time 1
	-0.40	-0.02			Time 1 + 5
Flowcell door glass without hours substrate wafer after process (position 3)	-0.70	+0.20			Time 1
	+0.40		-0.02		Time 1 + 5
Flowcell insert (position 4)	+0.02		+0.01		Time 1
	-0.02	+0.01	-0.01		Time 1 + 5 hours

Figure 7 depicts the location of electrostatic charge measurements. The apparatus 701 contains a load paddle 702, a flowcell 703, an unload paddle 705 and a flowcell insert 704. Measurements were taken in four locations, see Figure 7, locations 1, 2, 3, and 4 where significant electrostatic intensities were

previously observed. Reference measurements were also taken on a process development instrument located in another laboratory. Comparison of the numbers shows that the electrostatic charge can vary greatly both from one apparatus to another as well as on the same apparatus at different time points. The data in Table 2 show the intensities measured after ion emitter fans were installed on the instruments. Again the locations of the measurements refer to the locations in Figure 7. Model 6440 and 6430 ion emitter fans products, also from Ion Systems, Inc. were used to neutralize the electrostatic fields.

Table 2
Electrostatic Measurements after Ion Fan Installation (kV/inch)

Location		Apparatus A	B	C
Wafer on load paddle before process (position 1)				
	Start charge level	+1.33		+1.35
+1.40				
	Ending charge level	0.00	-0.02	+0.02
	Time (seconds)	8	5	5
Wafer on unload paddle after process (position 2)				
	Start charge level	+1.40	+1.40	+1.44
	Ending charge level	0.00	-0.01	
+0.01				
	Time (seconds)	4	5	5
Flowcell insert (position 4)				
	Start charge level	+1.41		+1.40
+1.46				
	Ending charge level	-0.02	-0.01	+0.02
	Time (seconds)	4	5	5

Fieldmeter readings taken during the synthesis process on all three instruments show the ion emitter fans can effectively maintain static field levels at +/- 0.01kV. Using a charge plate attached to the field meter, a 1-1.5kV

charge was applied and inserted into the ion flow to observe how quickly charges can be neutralized. All measurements show similar and consistent instrument performance. Steady state intensities level off at +/- 0.03 kV which are similar to background measurements.

It is believed that a deeper flow cell allows for better reagent mixing which results in greater synthesis uniformity. Table 3 summarizes the results of hybridization using 50nM oligonucleotide target sequences under standard conditions of 30 minutes at 25C. There is a noted improvement in both the average signal intensity and the corresponding chip coefficient of variation upon going to the 20 ml flow cell and using the 20mM phosphoramidite concentration, coupled with a modular oligo synthesizer (MOS) cycle which features replenish coupling (see Replen. Couple in Table 3) where reagents are added, allowed to mix and then more of the same reagents are added before the cycle is completed to ensure complete reagent mixing during the cycle.

Table 3
Flow Cell Density and Phophoramidite Concentration

Substrate	Flowcell Depth	[amid.]	Replen. Couple	Intensity	St. Dev.	%CV
A	0.010"	10 mM	No	16987.0	823.2	4.8%
B	0.010"	10	No	15466.0	1355.6	8.8%
C	0.020"	10	No	18381.9	663.5	3.6%
D	0.020"	10	No	22089.0	1082.5	4.9%
E	0.020"	10	Yes	23266.8	442.2	1.9%
F	0.020"	20	Yes	25643.7	772.3	3.0%

Wafers were synthesized under standard protocol conditions and then deprotected with methylamine [MET] (40% wt in H_20) for 8 hours. The deprotected wafers were then diced, assembled and hybridized under standard protocol conditions. These wafers were analyzed and the results were compared with identically processed wafers deprotected with Ethylenediamine [EDA](50% wt in EtOH). The MET wafers demonstrated a 110% increase in foreground

probe intensity, a 58% decrease in background probe intensity, and a 130%-400% increase in control probe intensity.

All publications and patent applications cited above are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent application were specifically and individually indicated to be so incorporated by reference. Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of fabricating an array comprising:

fabricating materials on a surface of a substrate to form an array of materials; and

removing static charge during said fabricating step.

2. The method of claim 1 wherein said fabrication step occurs in a reactor system.

3. The method of claim 2 wherein said reactor system comprises a flow cell.

4. The method of claim 1 wherein said array is a biological array.

5. The method of claim 1 wherein said materials are inorganic compounds.

6. The method of claim 1 wherein said materials are organic compounds.

7. The method of claim 1 wherein said materials are polymers.

8. The method of claim 7 wherein said polymers comprise nucleic acids.

9. The method of claim 1 wherein said removing static charge step is accomplished by an ionizing fan.

10. The method of claim 9 wherein said ionizing fan is a portable fan.

11. The method of claim 9 wherein said ionizing fan is permanently installed.

12. The method of claim 11 wherein said ionizing fan is installed in a ceiling.

13. A method of fabricating an array comprising:
manipulating the static charge on a reactor;
and fabricating materials using said reactor.

14. A method of removing protective groups from nucleotides comprising deprotecting said nucleotides with an alkylamine diluted in H_2O.

15. The method of claim 14 wherein said nucleotides are attached to a solid support.

16. The method of claim 15 wherein said solid support is a spatially-addressable array.

17. The method of claim 16 wherein said nucleotides comprise a plurality of oligonucleotide.

18. The method of claim 17 wherein said oligonucleotide are synthesized on said array.

19. The method of claim 14 wherein said removing protective groups is a deprotection step in an array fabrication process.

20. The method of claim 14 wherein said deprotection step lasts between 2 minutes and 20 hours.

21. The method of claim 14 wherein said deprotection step lasts between 1 hour and 10 hours.

22. The method of claim 14 wherein said deprotection step lasts between 4 hours and 8 hours.

23. A method of array fabrication comprising:

performing a photolysis step on a substrate, wherein said substrate comprises functional groups on its surface, wherein said photolysis step takes place inside a flow cell, said flow cell having a depth of between 0.100" and 0.005"; and

performing a chemical reaction, wherein said chemical reaction take place inside said flow cell.

24. The method of claim 23 wherein said depth is between 0.05" and 0.005".

25. The method of claim 23 wherein said depth is between 0.032" and 0.010".

26. The method of claim 23 wherein said depth is about 0.020".

27. The method of claim 23 wherein said flow cell has a width of 5" and a length of 5".

28. The method of claim 23 wherein said chemical reaction comprises:

addition of a 3'-O-activated phosphoramidited nucleoside (phosphoramidite) photoprotected at the 5' hydroxyl wherein said phosphormidite is present at a concentration of from mm to about 100mM.

29. The method of claim 28 wherein said phosphoramidite is present at a concentration of from 10mM to about 50mM.

30. The method of claim 28 wherein said phosphoramidite is present at a concentration of from 15mM to about 30mM.

31. The method of claim 28 wherein said phosphoramidite is present at a concentration of about 20mM.

32. The method of claim 23 wherein said flow cell has been manufactured such that shims and the flow cell body form a contiguous surface where the substrate contacts said flow cell body.

33. The method of claim 32 wherein said contiguous surface is formed by machining a groove into said body such that said shim fits into said groove.

34. The method of claim 32 wherein said shim encloses an o-ring.

35. A method of array fabrication comprising:

performing a photolysis step on a substrate, wherein said substrate comprises functional groups on its surface, and

performing a chemical reaction, wherein said chemical reaction take place inside said flow cell, wherein said chemical reaction comprises addition of a 3'-O-activated phosphoramidite nucleoside (phosphoramidite) photoprotected at the 5' hydroxyl wherein said phosphormidite is present at a concentration of from mm to about 100mM.

36. The method of claim 35 wherein said phosphoramidite is present at a concentration of from 10mM to about 50mM.

37. The method of claim 35 wherein said phosphoramidite is present at a concentration of from 15mM to about 30mM.

38. The method of claim 35 wherein said phosphoramidite is present at a concentration of about 20mM.

39. A method of array fabrication comprising:

performing a photolysis step on a substrate, wherein said substrate comprises functional groups on its surface, and

performing a chemical reaction, wherein said chemical reaction take place inside said flow cell wherein said flow cell has been manufactured such that the shims and the flow cell body form a contiguous surface where the substrate contacts said flow cell body.

40. The method of claim 39 wherein said contiguous surface is formed by machining a groove into said body such that said shim fits into said groove.

41. The method of claim 39 wherein said shim encloses an o-ring.

42. The method of claim 13 where said manipulating step comprises placing static removing equipment at entry and exit points of said reactor.

43. The method of claim 42 wherein said static removing equipment comprises an ionizing fan.

44. The method of claim 42 wherein said static removing equipment comprises an ion bar.

45. The method of claim 42 wherein said static removing equipment comprises an arrangement of at least one ionizing fan and at least one ion bar.

46. The method of claim 39 further comprising:

placing static removing equipment at entry and exit points of said flow cell.

47. The method of claim 46 wherein said static removing equipment comprises

an ionizing fan.

48. The method of claim 46 wherein said static removing equipment comprises an ion bar.

49. The method of claim 46 wherein said static removing equipment comprises an arrangement of at least one ionizing fan and at least one ion bar.

50. The method of claim 3 wherein at least one surface of said flow cell is made of stainless steel.

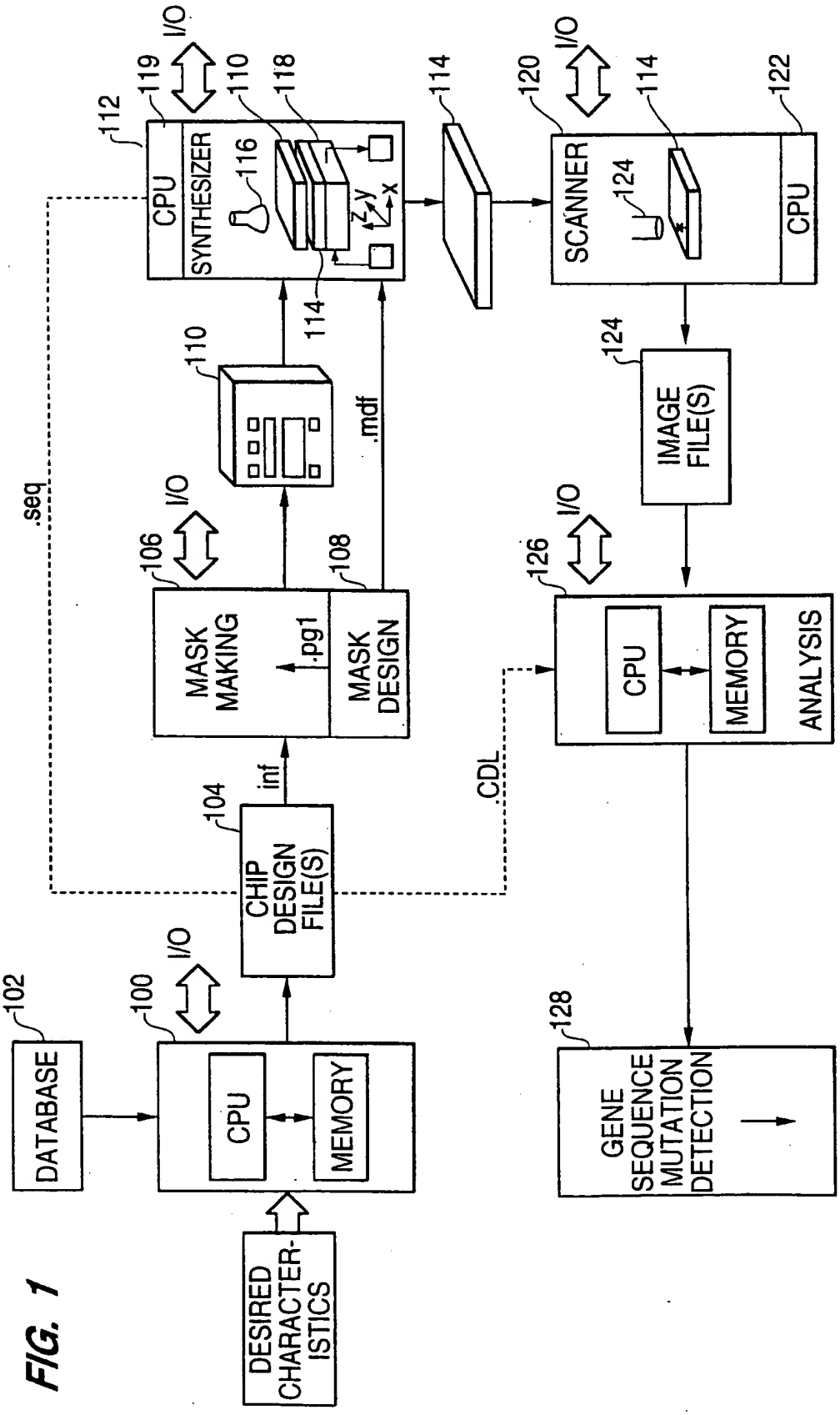
51. The method of claim 3 wherein at least one surface of said flow cell is made of glass.

52. The method of claim 51 wherein said glass is borosilicate glass.

53. The method of claim 23 wherein at least one surface of said flow cell is made of stainless steel.

54. The method of claim 23 wherein at least one surface of said flow cell is made of glass.

55. The method of claim 54 wherein said glass is borosilicate glass.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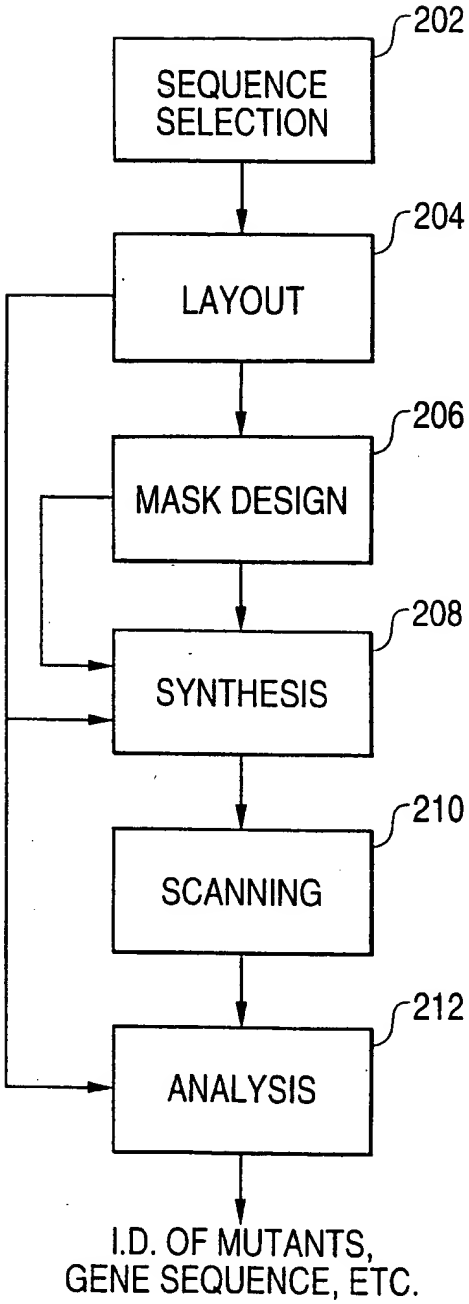
ANALYSIS
GENE SEQUENCE MUTATION DETECTION

FIG. 1

FIG. 2a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SCANNING
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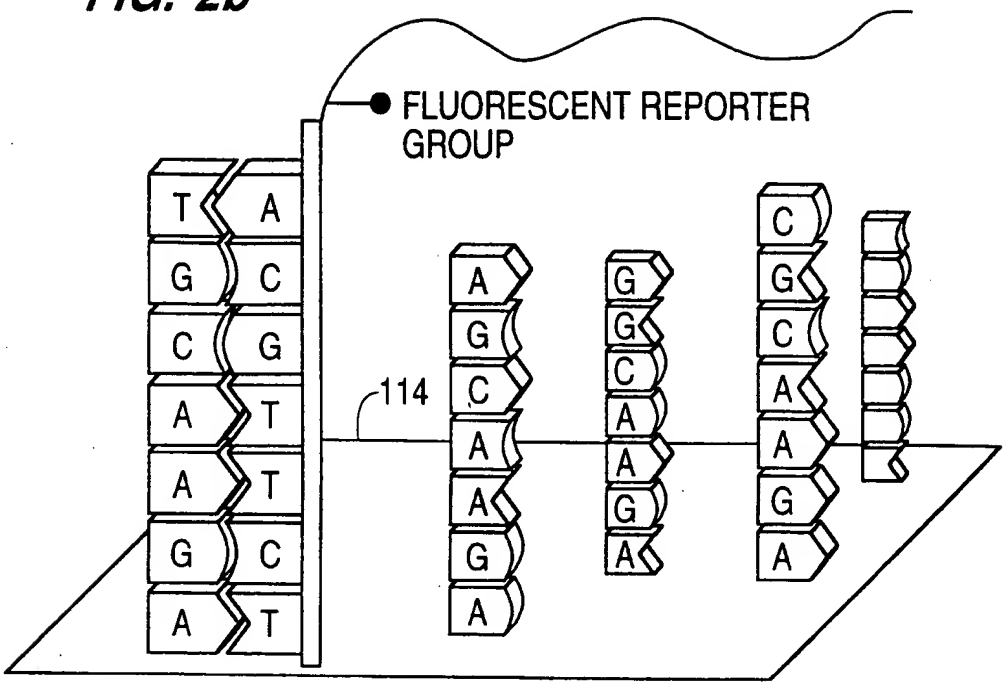
ANALYSIS
I.D. OF MUTANTS, GENE SEQUENCE, ETC.

FIG. 2b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T A
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FIG. 3

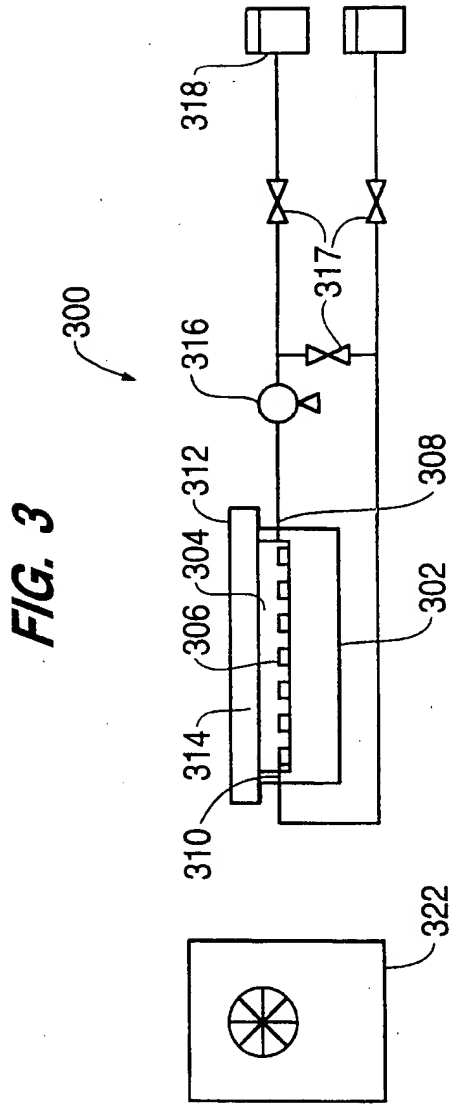

FIG. 4

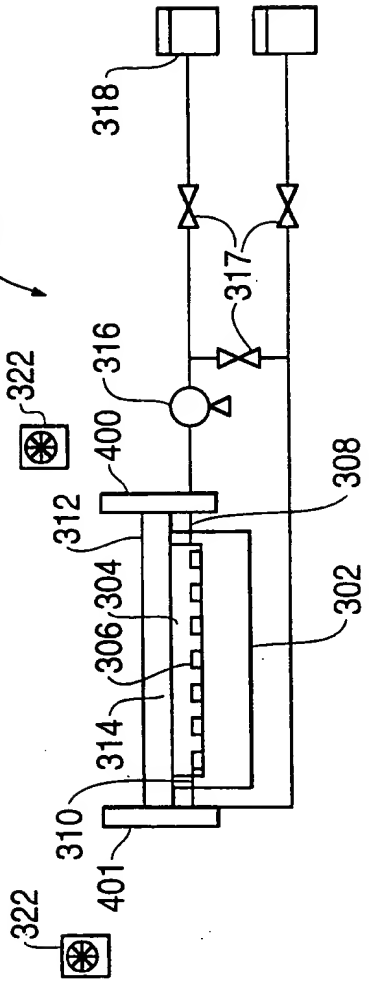

FIG. 5

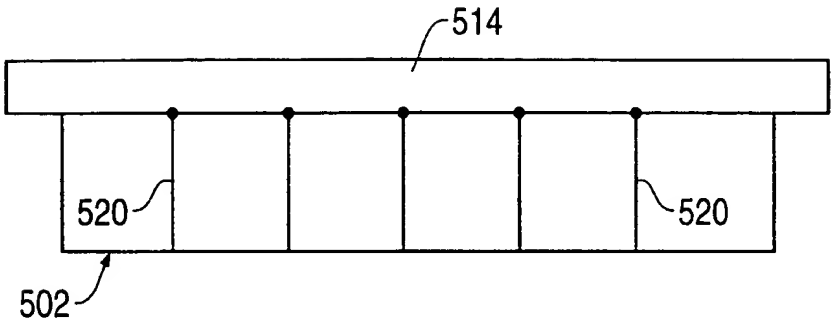

FIG. 6

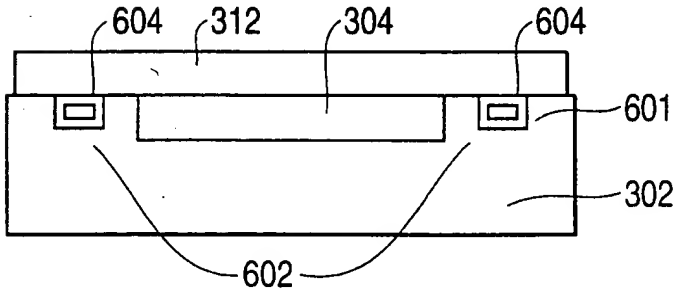

FIG. 7